The present amendments to claims 1, 2, 9, 25, 39, 53, 59, 60, 63, and 64 are fully supported by the application including the Drawings and claims as originally filed.

In particular, amended claim 1 is supported by disclosure on page 59, line 7 to page 60, last line (disclosing single primer amplification where the target template has inverted repeat sequences). See also Figure 3. Recitation of the word "suppressed" finds particular support on page 21, lines 12-18 (teaching suppression of template amplification).

As amended, claims 2, 9, 25, 39, 53, 59, and 60 find particular support on page 43, line 20 to page 45, line 4 (providing for specific polymerases having 3' to 5' exonuclease activity). See also Example 1, at page 75, lines 15-16 (disclosing use of Pfu DNA polymerase having the exonuclease activity).

Claims 63 and 64 were amended to improve claim language.

The amendments to claims 1, 2, 9, 25, 39, 53, 59, 60, 63, and 64 are intended to advance prosecution and should not be construed as surrender of any subject matter. Applicant reserves the right to file additional applications embracing such subject matter at a later date.

The present claim amendments do not introduce any new matter.

The specification was objected to on grounds that it includes reference to U.S. Patent applications whose status has changed yet is not so indicated. Applicant has updated the specification as requested Should the Examiner know the status of any application not updated, the undersigned would be most grateful to have that information.

Claims 1-57 and 69-65 stand rejected under 35 USC §112, first paragraph. In formulating the rejection, the position was taken on page 3 of the Office Action that:

> The method of claim 1 requires the presence of but one primer, yet requires one to make copies of a primer extension product seemingly using the same primer. The specification does not reasonably provide enablement for making copies of primer extension reaction products when but a single primer is present. The specification also does not provide an enabling disclosure whereby any type of "controlled" condition for elongating a primer that had a 3'-mismatch.

Although Applicant respectfully disagrees with the rejection, basis for it has been addressed by this submission.

In particular, amended claim 1 recites a target polynucleotide with inverted repeat structures. Those repeat structures are taught to assist formation of target sequence copies when one primer is used. See e.g,. page 59, line 7 to page 60, last line as well as Figure 3 (disclosing single primer amplification of target polynucleotide with inverted repeat structures PBNS1 and S2).

Further, claim 1 has been amended to recite "suppressed" instead of "control". That is, intended extension of the oligonucleotide primer along the second polynucleotide is suppressed (or reduced) relative to extension of that primer along the target sequence.

The Office Action further stated at pages 3-6 that:

> Claims 2-57 and 59-65 are not enabled by the specification for the amplification of any target nucleic acid wherein said method one also incorporates an internal control whose nucleotide residue sequence is such that it results in a 3'-mismatch on a primer that anneals thereto. Neither the written description nor the claims recite the conditions nor steps required for the detection of point mutations where one would obtain but a single nucleotide extension to a primer annealed to the target yet would remain unchanged after the use of 3'-5' exonuclease. Further, the method does not set forth a repeatable procedure where one is to perform multiplex PCR and a 3'-5' exonuclease is used while in the presence of a variety of primers. The very presence of a 3'-5' exonuclease in a sample will result in the degradation of primer(s), template, internal control, as well as any amplification product.
>
> It is well known in the art that hybridization and amplification reactions can and do incorporate the use of an array of primers or probes wherein said primers and/or probes are immobilized on the surface of a solid support. The claimed method encompasses such embodiments. The specification fails to address in sufficient detail how one would be

able to eliminate only the 3' terminal residues of a mismatched primer to a internal control when the same exonuclease can degrade the primers/probes on the surface of a array device, thereby allowing for the elongation of non-target sequences and the creation of conditions whereby primers previously non-complementary to a non-target sequence in the solution now become sufficiently complementary so to permit annealing and primer extension.

As disclosed by Sommer and Tautz, weak priming was achieved with as little as two 3' bases being complementary while successful priming was achieved in primers of 17-22 nucleotides in length where there were but 3 residues complementary. In view of the art recognizing this sensitivity to priming with a tremendous level of non-complementarily between the primer and target, the use of an exonuclease on a mixture of primers may well result in the amplification of the internal control, but would also result in the amplification f innumerable nontarget sequences as well. The specification has not provided a reproducible method whereby one would be able to prohibit such unwanted primer elongation and to differentiate between desired and non-desired product(s).

In order to practice the claimed method to the fullest extent of the claims' scope, the ordinary artisan would have to resort to the testing and evaluation of innumerable conditions and parameters with little, if any, reasonable expectation of success. Further, the claimed invention relates directly to matters of physiology and chemistry which are inherently unpredictable and as such, require greater levels of enablement.

[citations omitted]

While argument has been presented that polymerase chain reaction is well known in the art, and there is no need for additional guidance, the record does not reflect that the use of a 3'-5' exonuclease in a PCR reaction was well known in the art, especially when this enzyme has the capacity to destroy the amplified target, any primers as well as the positive control. Accordingly, the state of the prior art does not reflect that this modification to the conditions is routine. For the above reasons, and in the absence of convincing evidence to the contrary, the rejection is maintained and has been broadened so to encompass newly added claims.

While Applicant respectfully disagrees with the rejection, grounds for it have been addressed as follows.

As disclosed throughout the specification, a 3' end primer mismatch permits regulation of amplification of one polynucleotide relative to the another. See e.g., page 11 of Applicant's

specification. The mismatch helps to suppress amplification of the control template while providing good amplification of the target.

As understood, the rejection contends that the specification does not satisfy §112, first paragraph because certain primers may not amplify appropriately. Particular concern was raised about primers with 3' end mismatches that may amplify (citing Sommer and Tautz (*Nuc. Acids Res.* 17: 6749 (1989)).

Applicant respectfully disagrees with this ground of rejection for several reasons.

First, it is submitted that the instant specification fully satisfies the "how to make" and "how to use" requirements of §112, first paragraph.

More particularly, it is submitted that one working in this field having read the instant application could readily identify any primers that did not suitably amplify in accord with the invention. The level of skill in this particular field is high and no undue experimentation would be needed to identify, select and use appropriate primers to practice the invention.

The art of record exemplifies such skill in the field.

For example, the cited Sommer and Tautz article shows that amplification characteristics of many different primer/template combinations can be readily determined. See Table I of Sommer and Tautz (showing amplification of primers a-n). Primers described in the article include those with 3' end mismatches and/or internal non-complementarity. Significantly, the cited article reports success in segregating the primers into discreet categories ie., good (++), weak (+), or no amplification (-). See Table 1 of Sommer and Tautz. Thus as relied on, the article provides objective evidence that one working in the field could readily identify appropriate primers and primer/template combinations for use in accord with this invention.

More particularly, the cited Sommer and Tautz article shows that one working in this field could select primers that amplify well (++) and not so well (- or +) on specific templates. Also exemplified is that one could readily determine the amplification strength of primers having 3' end mismatches and /or internal non-complementarities on many different templates. See Table I of the Sommer and Tautz article.

Accordingly, the art of record in this case shows that a worker could readily select appropriate primers for use with the invention without engaging in any undue experimentation. If undesired amplification resulted with some primers ie., weak or non-specific priming on a particular control template, that primer could be identified and set aside (if needed) in favor of other more suitable primers.

As disclosed throughout Applicant's specification, particular primers of interest have a 3' mismatch which does not amplify well on the control template. Preferably, such primers amplify much better on the corresponding target template. No undue experimentation would be needed to identify and use such primers.

As mentioned, it is an object of the invention to permit amplification regulation of one polynucleotide relative to the another. See the Summary of the Invention, page 10, line 16 to page 11, line 9. Thus, if any subject primer gave weak control template amplification it would be useful in some embodiments if it gave better amplification on the corresponding target template.

Reconsideration and withdrawal of this ground of rejection are respectfully requested.

The §112, first paragraph, rejection also took the position that 3' to 5' exonuclease "will result in degradation of primer(s), template, internal control, as well as any amplification product". See Office Action at pages 3 and 6. Although Applicant respectfully disagrees with this contention, grounds for it have been addressed by this submission.

In particular, claims 2, 25, 39, and 53 have been amended to point out that the intended 3' to 5' exonuclease is a polymerase activity. Polymerases having that activity are known and often used in the field to amplify templates. Significant degradation of primers, templates and amplified products does not usually result. See e.g., Applicant's specification at page 44, line 19 to page 45, line 4 (disclosing particular polymerases having 3' to 5' nuclease activity). See also Example 1.

In view thereof, reconsideration and withdrawal of the §112, first paragraph rejection are requested.

In view of the discussion above, it is respectfully submitted that all rejections of record have been addressed. Early consideration and allowance of the pending claims are earnestly solicited. However if the Examiner feels that this response does not address all outstanding rejections of record, the undersigned would greatly appreciate the opportunity to discuss same in a telephone interview as soon as convenient.

The USPTO is hereby authorized to charge our deposit account no. 04-0010 for any fees necessary to consider this submission.

Respectfully submitted,

Date: 6/27/00

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